DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY INVERTEBRATES, FRESHWATER
OPPTS Guideline 850.1735

1. **CHEMICAL:** Cypermethrin PC Code No.: 109702

2. **TEST MATERIAL:** Cypermethrin Technical 40/60 Purity: 40.6% cis/59.4% trans

3. **CITATION:**

Authors:	Picard, C.R.
Title:	10-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to Cypermethrin Applied to California Sediment 2 Under Static-Renewal Conditions.
Study Completion Date:	May 11, 2009
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571
Sponsor:	Pyrethroid Working Group Beverage & Diamond 1350 I Street NW Washington, DC 20005
Laboratory Report ID:	13656.6127
MRID No.:	47946603
DP Barcode:	420006

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: Christie E. Padova **Date:** 06/08/10

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers **Date:** 06/11/10

5. **APPROVED BY:** Stephen Carey, Biologist, OCSPP/EFED/ERB6

Signature: Stephen Carey **Date:** 7/21/15

6. **STUDY PARAMETERS:**

Age of Test Organism:	7 to 8 days old
Definitive Test Duration:	10 days
Study Method:	Intermittent flow-through
Type of Concentrations:	Mean-measured

7. CONCLUSIONS:

Results Synopsis:

Based upon mean-measured sediment concentrations:

Survival:
LC_{50}: 28 µg a.i./kg — 95% C.I.: 20 to 43 µg a.i./kg
Slope: 4.15 (1.86 to 6.44)
NOAEC: 16 µg a.i./kg
LOAEC: 32 µg a.i./kg

Growth:
EC_{50}: 30 µg a.i./kg — 95% C.I.: 20 to 46 µg a.i./kg
Slope: 1.94±0.534
NOAEC: 9.1 µg a.i./kg
LOAEC: 16 µg a.i./kg

Based upon ESTIMATED[1] pore water concentrations:

Survival:
LC_{50}: 0.007 µg a.i./L — 95% C.I.: 0.005 to 0.01 µg a.i./L
Slope: 4.15 (1.86 to 6.44)
NOAEC: 0.004 µg a.i./L
LOAEC: 0.008 µg a.i./L

Growth (dry weight):
IC_{50}: 0.007 µg a.i./L — 95% C.I.: 0.005 to 0.01 µg a.i./L
Slope: 1.94±0.534
NOAEC: 0.002 µg a.i./L
LOAEC: 0.004 µg a.i./L

Based upon OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 930 µg a.i./kg TOC — 95% C.I.: 670 to 1400 µg a.i./kg TOC
Slope: 4.15 (1.86 to 6.44)
NOAEC: 530 µg a.i./kg TOC
LOAEC: 1100 µg a.i./kg TOC

1 Freely dissolved pore water endpoints (ug/L) estimated as:
Mean measured bulk sediment conc. (*ug/kg-dw*) / [Fraction TOC (*kg OC/kg-dw*) * K_{OC} (*L/kg-OC*)]

Growth (dry weight):
EC_{50}: 1000 µg a.i./kg TOC 95% C.I.: 670 to 1500 µg a.i./kg TOC
NOAEC: 300 µg a.i./kg TOC
LOAEC: 530 µg a.i./kg TOC

8. ADEQUACY OF THE STUDY:

A. Classification: Acceptable

B. Rationale: N/A

C. Repairability: N/A

9. MAJOR GUIDELINE DEVIATIONS:

No major deviations noted.

10. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *H. azteca* or *Chironomus tentans*	*Hyalella azteca*
Life Stage: For *C. tentans*: third instar (9-11 days old). The instar stage of midges must be confirmed by head capsule width (approx. 0.38 mm). For *H. azteca*: 7- to 14-day old amphipods must be produced. If growth is also an endpoint, a narrower range, such as 1- to 2-day old amphipods should be used.	7 to 8 days old
Supplier Brood stock can be obtained from laboratory, commercial, or government sources. (Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	Amphipods originated from laboratory cultures maintained in *ca.* 15 L of culture water (same source as dilution water) under flow-through conditions.

Guideline Criteria	Reported Information
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period: The required culture and testing temperature is 23°C. The test organisms should be cultured in the same water to be used for testing.	Adults were removed from the main culture tanks 8 days prior to test initiation and placed in *ca.* 8 L of water. Juvenile amphipods (<24 hours old) produced by the isolated adults were then transferred to *ca.* 0.80 L of laboratory dilution water and reared under static conditions for 7 to 8 days with gentle aeration. During the holding period, the dissolved oxygen ranged from 7.9 to 8.3 mg/L and temperature ranged from 23 to 24 °C.
Feeding:	During holding and acclimation, amphipods were fed every other day with 2.5 mL of a combination of yeast, cereal leaves, and flaked fish food suspension (YCT) and 2.5 mL of *Ankistrodesmus falcatus*.
Pretest Mortality: A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test).	No mortality during the 48 hours prior to test initiation.

C. Test System

Guideline Criteria	Reported Information

Guideline Criteria	Reported Information
Source of dilution water (overlying water) and sediment: Soft reconstituted water or water from a natural source. Tap water is acceptable if it is dechlorinated, deionized, and carbon filtered, but its use is not encouraged. Uncontaminated natural sediment is recommended.	Laboratory well water characterized as having a total hardness and total alkalinity as $CaCO_3$ of 44 to 46 and 21 to 22 mg/L, respectively, a pH range of 7.1 to 7.2, and a specific conductance of 330 µmhos/cm. Monthly analysis of the water source indicated a TOC 0.33 mg/L for April 2009. Natural sediment (Batch No. EMP0073) collected on November 21, 2008 from the Sacramento-San Joaquin Delta, CA and designated as CA Sediment 2. The sediment was wet-pressed sieved (2.0-mm) prior to use.
Does water support test animals without observable signs of stress?	Yes.
Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L	There were no apparent problems with water quality. During the study, ammonia levels (as N) in the overlying water were ≤0.70 mg/L.
Water Temperature 23°C for both species. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1°C and 3°C, respectively.	Daily: 23 to 25°C Continuous: 23 to 24°C
pH Should not vary more than 50%. Survival is best at pH >6.5 for *C. tentans*..	6.9 to 7.4
Dissolved Oxygen Maintained between 40 and 100%.	5.8 to 7.6 mg/L (≥69% ASV at 24°C; reviewer-calculated)

Guideline Criteria	Reported Information
Total Hardness Should not vary more than 50%. *H. azteca* are sensitive to hardness (e.g., they are not found in waters with calcium at <7 mg/L and DO at <2 mg/L).	48 to 76 mg/L as $CaCO_3$
Conductivity Should not vary more than 50%.	320 to 380 µmhos/cm
Sediment Characterization All sediment must be characterized for: pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content.	Particle distribution – 53% sand, 20% silt, 27% clay (sandy clay loam; reviewer-derived from USDA soil texture triangle) Organic carbon content – 3.0% Solids – 48.65% pH – 7.2 Ammonia concentration of pore water – not reported
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	None reported
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	<u>Cypermethrin Technical 40/60</u> Synonym: FMC 30980 IUPAC Name: (*RS*)-α-cyano-3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate CAS Name: cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate CAS No.: 52315-07-8 Description: not reported Lot No.: PL07-0633 Purity: 40.6% cis-isomer, 59.4% trans-isomer Storage: dark, room temperature

Guideline Criteria	Reported Information
Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.	Two hundred (200) mL of a 20-µg a.i./L stock solution was prepared in acetone. From this, five individual dosing solutions were prepared by diluting the appropriate amount of stock solution with acetone to 10 mL. All stock and dosing solutions were clear and colorless, with no visible un-dissolved test substance. Negative and solvent controls were included in the test.
Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.	A jar-rolling technique was used to apply the test substance to the sediment. A 9-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.050 kg of fine silica sand in glass Petri dishes, and the solvent was allowed to evaporate off for 30 minutes. The dry sand was then added to 3.5 kg of wet sediment (total of 1.753 kg dw) in individual 1-gallon jars. Each jar was then rolled for 4 hours at room temperature at approx. 15 rpm. The jars were stored upright at 2 to 8°C during conditioning. The treated sediments were allowed to equilibrate for a 14-day period in the refrigerator. Twice a week during the conditioning period and prior to addition to the exposure vessels (day -1), the jars were mixed on the rolling mill for an additional 2 hours at room temperature to ensure the sediment was homogeneous. The range of concentrations (5.0 to 80 µg a.i./kg) was based upon the results of a preliminary range finding study.

Guideline Criteria	Reported Information
Test Aquaria 1. Material: Glass or stainless steel or perfluorocarbon plastics. 2. Size: 300 ml high-form lipless beakers containing 100 ml of sediment and 175 ml of overlying water.	300-mL glass vessels containing 100 mL (approx. 4.0-cm layer) of sediment (equivalent to 52 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at *ca.* 275 mL. Test vessels were covered with 40-mesh Nitex® screen for drainage.
Type of Dilution System Daily renewal or a flow-through system may be used.	Intermittent flow-through
Flow Rate 2 volume changes/day	2 volume additions/day
Aeration Dilution water should be vigorously aerated prior to use so that dissolved oxygen in the overlying water remains above 40% saturation.	None reported
Photoperiod 16 hours light, 8 hours dark at 500 to 1000 lux.	16 hours light, 8 hours dark; 550 to 690 lux
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used,it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Acetone, 9 mL per 1.753 kg dw sediment. The acetone was allowed to completely evaporate during the mixing procedure.

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers	

Guideline Criteria	Reported Information
One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	One day prior to the addition of amphipods (day -1), the test systems were established. Overlying water was gently added, and each vessel was placed under the renewal system.
Renewal of Overlying Water: Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.	The overlying water was replaced twice daily using an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least twice daily for proper functioning.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	Amphipods were impartially assigned one or two at a time into intermediate test beakers until all beakers contained ten amphipods. The test was initiated when each intermediate beaker of amphipods was added to each respective test vessel.
Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment**.**	<u>Preliminary toxicity assessment</u> • Treated sediment equilibrated for 8 days • 10-day exposure at nominal levels of 0 (negative and solvent controls), 0.010, 0.10, 1.0, 10, and 100 µg a.i./kg • three replicates per level, each containing 10 organisms • Survival averaged 87 (negative control), 90 (solvent control), 100, 97, 97,67, and 0%, respectively • Dry weight averaged 0.08 (negative control), 0.09 (solvent control), 0.08, 0.08, 0.08, and 0.06 mg, respectively
Monitoring the test All test chambers should be checked daily and	Test vessels were observed daily for

Guideline Criteria	Reported Information
observations made to assess organism behavior such as sediment avoidance.	mortality and abnormal behavior.
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	0 (negative and solvent controls), 5.0, 10, 20, 40, and 80 µg a.i./kg sediment
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	80 amphipods per level, with 10 amphipods per replicate vessel and 8 biological replicates per level An additional 20 replicates were maintained for chemical analysis
Test organisms randomly or impartially assigned to test vessels?	Yes
Feeding *C. tentans* in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin7 suspension daily. *H. azteca* may be fed with a mixture of yeast, Cerophyl, and trout chow (YCT) at a rate of 1.5 mL daily per test chamber. A drop in DO levels below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until DO levels increase.	1.0 mL of yeast, cereal leaves, and flaked fish food suspension (YCT) once daily.

Guideline Criteria	Reported Information
Water Parameter Measurements Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of the test. DO should be measured daily. Temperature should be measured daily in one test chamber from each treatment. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 and 3°C, respectively.	<u>Overlying water:</u> For all levels, total hardness, alkalinity, specific conductance, and ammonia concentrations were measured in a composite sample on Days 0 and 10. DO, temperature, and pH were measured in each replicate vessel on Days 0 and 10 and in one alternating replicate from each level on Days 1 to 9. Temperature was also continuously monitored in an auxiliary vessel in the water bath. <u>Pore water:</u> Redox potential, pH, ammonia, and dissolved organic carbon (DOC) were measured in a composite sample on Days 0 and 10.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	Surrogate samples vessels were collected on Days 0 and 10, and concentrations of cypermethrin were determined in pore water and sediment (see Reviewer's Comments section). The sediment/pore water matrices were isolated by centrifuging for 15 to 30 minutes at 1200 *g*. Aliquots of the dosing stock solutions were analyzed for cypermethrin. In addition, treated sediment from all levels were analyzed for cypermethrin prior to the allocation of the sediment into the replicate vessels (following equilibration).

11. <u>REPORTED RESULTS</u>:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in accordance with GLP Standards as specified in 40 CFR 160 with the following exceptions: the routine water, sediment, and food contaminant screening analyses.
Control Criteria Was control mortality ≤20%? Were control *C. tentans* an average size of ≥0.6 g?	<u>Mortality:</u> Negative control – 7% Solvent control – 1%
Percent Recovery of Chemical:	Procedural recoveries (QC samples) conducted concurrently with sample analysis: <u>Sediment:</u> 75.2 to 116% of nominal (original analysis) 78.4 to 107% of nominal (re-analysis) <u>Aqueous:</u> 91.3 to 112% of nominal (with one outlier of <LOQ)
Data Endpoints - Survival - Dry weight (determined by pooling all living organisms from a replicate and drying at 60 to 90°C to a constant weight) - Body length (amphipod only)	- Survival - Dry weight
Raw data included?	Yes, sufficient

Effects Data

Toxicant Concentration				Survival		Dry Weight	
Nominal (µg a.i./kg)	Mean Measured[a]						
	Sediment (µg a.i./kg dw)	Pore Water (µg a.i./L)	Overlying Water (µg a.i./L)	Mean %	% Inhibition	mg per larvae	% inhibition
Control	<LOQ	<LOQ	Not assessed	93	N/A	0.10	N/A
S. Control	<LOQ	<LOQ	Not assessed	99	-6	0.09	10
5.0	4.3	0.014	Not assessed	93	0	0.09	10
10	9.1	0.019	Not assessed	96	-3.2	0.11	-10
20	16	0.034	Not assessed	86	7.5	0.08*	20
40	32	0.050	Not assessed	28*	70	0.03[b]	70
80	59	0.13	Not assessed	13*	86	0.04[b]	60

[a] LOQ were equivalent to 0.21 to 0.22 µg a.i./kg for sediment samples and 0.0011 to 0.020 µg a.i./L for pore water samples.
[b] Excluded from statistical analyses due to significant effect on survival.
* Statistically different ($p \leq 0.05$) compared to the negative control.

Other Significant Results:

Biological: After 10 days, survival averaged 93 and 99% for the negative and solvent controls, respectively, and 93, 96, 86, 28, and 13% for the mean-measured 4.3, 9.1, 16, 32, and 59 µg a.i./kg sediment levels, respectively. Differences at the 32 and 59 µg a.i./kg levels were statistically-reduced ($p \leq 0.05$) compared to the negative control. The 10-day LC_{50} (with 95% C.I.) was reported by the study author to be 27 (25 to 28) µg a.i./kg sediment, and the NOAEC for survival was 16 µg a.i./kg.

After 10 days, dry weight averaged 0.10 and 0.09 mg per larvae at the negative and solvent control levels, respectively, and 0.09, 0.11, 0.08, 0.03, and 0.04 mg per larvae at the mean-measured 4.3, 9.1, 16, 32, and 59 µg a.i./kg sediment levels, respectively. The difference at the 16 µg a.i./kg sediment level was statistically-reduced ($p \leq 0.05$) compared to the negative control (the 32 and 59 µg a.i./kg levels were not statistically compared due to the significant effect on survival at these levels). The 10-day EC_{50} (with 95% C.I.) was reported to be 26 (23 to 29) µg a.i./kg sediment, and the NOAEC for amphipod growth was 9.1 µg a.i./kg.

Analytical: Concentrations of cypermethrin were determined on Days 0 and 10 in sediment and pore water only (see Reviewer's Comments section). At all except the lowest treatment level,

concentrations decreased in sediment and pore water during the 10-day study. In sediment, reviewer-calculated percent changes were +2.3, -20, -40, -40, and -43% from Days 0 to 10 at the nominal 5.0, 10, 20, 40, and 80 µg a.i./kg sediment levels, respectively. Mean-measured sediment concentrations were 4.3, 9.1, 16, 32, and 59 µg a.i./kg sediment, representing 87, 91, 81, 80, and 74% of the nominal treatment levels, respectively. In pore water, reviewer-calculated percent changes were +15, -42, -49, -51, and -41% from Days 0 to 10 at the nominal 5.0, 10, 20, 40, and 80 µg a.i./kg sediment levels, respectively.

Nominal Sediment Concn. (µg a.i./kg)	Sediment, µg a.i./kg		Pore Water, µg a.i./L		Overlying Water	
	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10
Control	<0.21	<0.22	<0.0015	<0.020	Not assessed	Not assessed
S. Control	<0.21	<0.22	<0.0011	<0.020	Not assessed	Not assessed
5.0	4.3	4.4	0.013	0.015	Not assessed	Not assessed
10	10	8.0	0.024	0.014	Not assessed	Not assessed
20	20	12	0.045	0.023	Not assessed	Not assessed
40	40	24	0.068	0.033	Not assessed	Not assessed
80	75	43	0.17	0.10	Not assessed	Not assessed

B. Statistical Results

Statistical analyses were performed on amphipod survival and growth (dry weight). Analyses were performed using the response values for each replicate test vessel within a treatment level. Percent survival data were arcsine square-root transformed prior to analysis.

A t-Test was used to compare the performance of the negative control and solvent control data. A statistically-significant difference was indicated for survival; however, survival for both the control and solvent control groups was ≥90% and differences observed between the two groups were within the range of natural variability. Control performance for growth was statistically similar. All treatment groups were compared to the negative control data to determine treatment-level effects.

Normality of the data was evaluated using the Chi-Square Test, and homogeneity of variance was evaluated using Bartlett's Test. Data met both assumptions and were thus analyzed using Dunnett's and Bonferroni's Tests at the 95% level of certainty. NOAEC and LOAEC values were assigned based upon significance.

The linear interpolation method was used to calculate the LC/EC_{50} values with associated 95%

confidence intervals.

Analyses were performed using TOXSTAT Version 3.5 statistical software and mean-measured sediment concentrations.

Survival:
LC_{50}: 27 µg a.i./kg 95% C.I.: 25 to 28 µg a.i./kg
NOAEC: 16 µg a.i./kg
LOAEC: 32 µg a.i./kg

Growth:
EC_{50}: 26 µg a.i./kg 95% C.I.: 23 to 29 µg a.i./kg
NOAEC: 9.1 µg a.i./kg
LOAEC: 16 µg a.i./kg

12. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer statistically analyzed data for day 10 survival and dry weight. For both endpoints the negative and solvent control data were compared using a Student's t-test; for survival, a significant promotion ($p<0.05$; 6%) was detected in the solvent control, relative to the negative control. However, because survival was 99% in the solvent control and 93% in the negative control, solvent interference was not suspected to have played a role in this study. The data for survival and dry weight were further tested using Shapiro-Wilk's test to confirm normality and using Levene's test to confirm homogeneity of variances. The 32 and 59 µg a.i./kg dry weight data were excluded from this analysis, due to significant effects on survival at these levels. Survival and dry weight data satisfied the assumptions of ANOVA, so the NOAEC and LOAEC were determined using this test, followed by Dunnett's (dry weight) or William's (survival; dose-dependent response) test. These analyses were conducted using Toxstat 3.5 statistical software. The LC_{50} and EC_{50} values were determined using the Probit method. For survival, the Probit method was run using Toxanal 2009 and selected over the other methods as the best for characterizing the data, despite the poor fit of the data to the model (Goodness of fit probability = 0.012). The Probit method used to obtain the EC_{50} for dry weight was run using Nuthatch statistical software.

All of the above statistical analyses were performed in terms of the mean-measured sediment and estimated pore water treatment concentrations. Sediment endpoints are also calculated on an organic carbon-normalized basis, based on the following equation using an average TOC of 3.0%:

$$\text{mg/kg OC} = \frac{\text{mg/kg dry weight}}{\text{kg TOC/kg dry weight}}$$

Based upon mean-measured sediment concentrations:

Survival:
LC_{50}: 28 µg a.i./kg — 95% C.I.: 20 to 43 µg a.i./kg
Slope: 4.15 (1.86 to 6.44)
NOAEC: 16 µg a.i./kg
LOAEC: 32 µg a.i./kg

Growth:
EC_{50}: 30 µg a.i./kg — 95% C.I.: 20 to 46 µg a.i./kg
Slope: 1.94±0.534
NOAEC: 9.1 µg a.i./kg
LOAEC: 16 µg a.i./kg

Based upon ESTIMATED[2] pore water concentrations:

Survival:
LC_{50}: 0.007 µg a.i./L — 95% C.I.: 0.005 to 0.01 µg a.i./L
Slope: 4.15 (1.86 to 6.44)
NOAEC: 0.004 µg a.i./L
LOAEC: 0.008 µg a.i./L

Growth (dry weight):
IC_{50}: 0.007 µg a.i./L — 95% C.I.: 0.005 to 0.01 µg a.i./L
Slope: 1.94±0.534
NOAEC: 0.002 µg a.i./L
LOAEC: 0.004 µg a.i./L

Based upon OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 930 µg a.i./kg TOC — 95% C.I.: 670 to 1400 µg a.i./kg TOC
Slope: 4.15 (1.86 to 6.44)
NOAEC: 530 µg a.i./kg TOC
LOAEC: 1100 µg a.i./kg TOC

Growth (dry weight):
EC_{50}: 1000 µg a.i./kg TOC — 95% C.I.: 670 to 1500 µg a.i./kg TOC
Slope: 1.94±0.534

2 Freely dissolved pore water endpoints (ug/L) estimated as:
Mean measured bulk sediment conc. (*ug/kg-dw*) / [Fraction TOC (*kg OC/kg-dw*) * K_{OC} (*L/kg-OC*)]

NOAEC: 300 µg a.i./kg TOC
LOAEC: 530 µg a.i./kg TOC

13. REVIEWER'S COMMENTS:

The reviewer's NOAEC and LOAEC conclusions for survival and dry weight agreed with those of the study author; however, the LC_{50} and EC_{50} estimates slightly differed due to the different methods used to obtain these values. The reviewer's results were obtained using EFED-approved statistical programs, so they are reported in the Conclusions section.

While the reviewer's analysis detected a significant ($p<0.05$; 6%) increase in survival of solvent control amphipods, compared to negative control amphipods, the reviewer does not believe that this is evidence of solvent interference. While not directionally the same, the magnitude of the effect is comparable to that in the treatment levels at and below the NOAEC, which were not statistically different from the negative control.

Results were provided in terms of mean-measured sediment (bulk and OC-normalized) and estimated pore water concentrations in the Conclusions section of the DER.

Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high K_{oc} values) and regular renewal of the overlying water. It was also reported that previous studies performed at the laboratory indicated that only negligible amounts of pyrethroids partition to overlying water (Springborn Smithers Laboratories Study Nos. 13656.6106, 13656.6107, 13656.6110, 13656.6111, and 13656.6112, Putt, 2005).

This reviewer notes that the concentration of cypermethrin measured in pore water likely reflects both "freely dissolved" chemical (i.e., chemical that is not sorbed onto particulate organic carbon (POC) or dissolved organic carbon (DOC) in addition to dissolved chemical that is sorbed to DOC. This finding is indicated by the fact that the extraction and analytical methods used in this study do not distinguish among the two phases of chemical (freely dissolved and DOC-sorbed). It is also indicated by the much higher measured concentrations of cypermethrin in pore water than would be expected based on estimated values using sediment cypermethrin concentrations, its Koc, and sediment total organic carbon (TOC). For highly hydrophobic chemicals like cypermethrin, DOC in pore water can substantially reduce its bioavailability and toxicity. It is further noted that the pore water estimated environmental concentrations (EECs) generated using the Agency's PRZM/EXAMS model are based on freely dissolved chemical. Therefore, some downward adjustment of these pore water toxicity values using appropriate methods (e.g., Koc and DOC concentration in pore water) will likely be needed when comparing these values to freely dissolved EECs generated using PRZM/EXAMS. Since the measured pore water concentrations of cypermethrin do not accurately describe the exposure to parent compound, endpoints from this study will not be expressed in terms of measured pore water concentrations.

Instead, this reviewer has estimated freely dissolved pore water endpoints based on

measured concentrations in bulk sediment, the fraction of total organic carbon in bulk sediment (3.0%) and the mean K_{OC} (141,700 L/kg-OC, MRID 42129002) for cypermethrin. These estimated pore water endpoints, which are based on the freely dissolved test material (i.e., chemical that is not sorbed onto particulate organic carbon [POC] or dissolved organic carbon [DOC]), are consistent with the expression of aquatic estimated environmental concentrations (EECs) from PRZM/EXAMS. It is noted, however, that K_{OC} values for cypermethrin vary considerably depending on soil type (20,800 – 328,500 L/kg). This range of K_{OC} likely reflects differences in organic carbon composition and other soil properties used to determine K_{OC}. Therefore, these estimated pore water endpoints are subject to the same uncertainty in determination and application of K_{OC} for cypermethrin.

Nominal Sediment (µg a.i./kg)	Mean-measured Sediment (µg a.i./kg)	Estimated Pore Water (µg a.i./L)	OC-Normalized Sediment (µg a.i./g OC)
5.0	4.3	0.0009	140
10	9.1	0.002	300
20	16	0.004	530
40	32	0.008	1100
80	59	0.01	1970

Analysis of the stock solution samples used to dose the test sediments ranged from 79 to 160% of nominal fortified concentrations. Pretest analysis of the spiked sediment following equilibration and prior to allocation into the replicate exposure vessels ranged from 86 to 100% of nominal concentrations.

In pore water (measured at each level on Days 0 and 10), the redox potential ranged from 180 to 300 mV, the pH ranged 7.2 to 7.6, the DOC ranged from 6.1 to 9.3 mg C/L, and the ammonia (as N) ranged from 1.5 to 5.9 mg/L.

The analytical method used to quantify cypermethrin in California sediment 2 was validated in April 2009. Fortified samples were extracted two times with 50:50 methanol:purified reagent water and hexane; the extracts were combined and purified for analysis using solid phase extraction (SPE). Aliquots were analyzed using gas chromatography equipped with mass selective detection in negative chemical ionization mode (GC-MS/NCI). In samples fortified at 0.100 and 100 µg/kg, recoveries averaged 101 ± 3.62% and 88.7 ± 1.63%, respectively, with a limit of quantitation (LOQ) of 0.0515 µg a.i./kg.

The analytical method used to quantify cypermethrin in freshwater was validated in January 2009. Fortified samples were acidified and extracted twice with ethyl acetate; the combined extracts were reduced in volume using rotary evaporation (30°C) and taken to dryness under nitrogen (room temperature). The residues were re-constituted in 0.1% peanut oil in acetone and analyzed using gas chromatography equipped with mass selective detection in negative chemical ionization mode (GC-MS/NCI). In samples fortified at 0.00100 (sample LOQ), 0.00300, 0.0200, and 0.0500 µg/L, recoveries averaged 114 ± 3.82%. Due to the low concentrations being tested, the LOQ

was set at 0.00100 µg/L; sample LOQ recoveries averaged 110 ± 16.1%.

It was reported that representative samples of the overlying water source were periodically analyzed for pesticides, PCBs, and toxic metals, and that none of these compounds were detected in any of the water samples analyzed in agreement with ASTM guidelines.

Definitive test dates were April 10 to 20, 2009.

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15. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
   Title:  Day 10 % Survival
   File:        6603s          Transform:         NO TRANSFORMATION

       t-Test of Solvent and Blank Controls         Ho: GRP1 Mean = GRP2 Mean
 ============================================================================
 GRP1 (Solvent cntl) Mean =    92.5000     Calculated t value =    -2.2361
 GRP2 (Blank cntl) Mean   =    98.7500     Degrees of freedom =    14
 Difference in means      =    -6.2500
 ============================================================================
 2-sided t value (0.05,14) = 2.1448**   Significant difference at alpha=0.05
 2-sided t value (0.01,14) = 2.9768  No significant difference at alpha=0.01

     WARNING:  This procedure assumes normality and equal variances!


     NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
      OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
      THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.


     ************************************************************************
     CONC.     NUMBER         NUMBER         PERCENT        BINOMIAL
               EXPOSED        DEAD           DEAD           PROB.(PERCENT)
      59        74             64             86.48649       0
      32        74             52.00001       70.2703        0
      16        74             5              6.7568         0
      9.100001                 80             3              3.75          0
      4.3       74             0              0              0

     BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT
     CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE
     UNRELIABLE.  USE THE INTERVALS CALCULATED BY THE OTHER TESTS.


     AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 26.2289


     RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
        SPAN     G           LC50           95 PERCENT CONFIDENCE LIMITS
      3        2.395598E-02    29.06696       25.98119      32.94061


     RESULTS CALCULATED USING THE PROBIT METHOD
     ITERATIONS             G              H             GOODNESS OF FIT
PROBABILITY
         5                   .3041615       3.641177
1.214659E-02

     SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
     USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.
```

```
   SLOPE   =               4.148022
   95 PERCENT CONFIDENCE LIMITS = 1.860353    AND    6.435691

   INTERCEPT=-6.027873

   LC50 =    28.39174
   95 PERCENT CONFIDENCE LIMITS =  19.81637 AND  42.73518

            LC25 =         19.5247
   95 PERCENT CONFIDENCE LIMITS =  10.75457 AND  26.84386

            LC10 =         13.9388
   95 PERCENT CONFIDENCE LIMITS =  5.496151 AND  19.9393

            LC05 =         11.39309
   95 PERCENT CONFIDENCE LIMITS =  3.595444 AND  17.07183

Title:  Day 10 % Survival
File:             6603s             Transform:             NO TRANSFORMATION

                  Shapiro - Wilk's Test for Normality
--------------------------------------------------------------------------------

     D = 3375.0000
     W =   0.9585

     Critical W  = 0.9290 (alpha = 0.01 , N = 48)
              W  = 0.9470 (alpha = 0.05 , N = 48)

--------------------------------------------------------------------------------

Data PASS normality test (alpha = 0.01). Continue analysis.



Title:  Day 10 % Survival
File:             6603s             Transform:             NO TRANSFORMATION

                  Levene's Test for Homogeneity of Variance

                               ANOVA Table
--------------------------------------------------------------------------------

   SOURCE             DF               SS                MS           F
--------------------------------------------------------------------------------
   Between             5            241.6667          48.3333       1.5321

   Within (Error)     42           1325.0000          31.5476
--------------------------------------------------------------------------------
   Total              47           1566.6667
--------------------------------------------------------------------------------
                                                        (p-value = 0.2006)
```

```
   Critical F  = 3.4882  (alpha = 0.01, df = 5,42)
               = 2.4377  (alpha = 0.05, df = 5,42)

   Since  F < Critical F  FAIL TO REJECT  Ho: All equal (alpha = 0.01)


  Title:  Day 10 % Survival
  File:             6603s             Transform:             NO TRANSFORMATION

                               ANOVA Table
  ------------------------------------------------------------------------------

     SOURCE             DF               SS                MS           F
  ------------------------------------------------------------------------------
     Between             5          56416.6667       11283.3333     140.4148

     Within (Error)     42           3375.0000          80.3571
  ------------------------------------------------------------------------------
     Total              47          59791.6667
  ------------------------------------------------------------------------------
                                                              (p-value = 0.0000)

     Critical F  = 3.4882  (alpha = 0.01, df = 5,42)
                 = 2.4377  (alpha = 0.05, df = 5,42)

     Since  F > Critical F  REJECT  Ho: All equal (alpha = 0.05)


  Title:  Day 10 % Survival
  File:             6603s             Transform:             NO TRANSFORMATION

        Dunnett's Test   -   TABLE 1 OF 2             Ho:Control<Treatment
  ------------------------------------------------------------------------------
                                TRANSFORMED    MEAN CALCULATED IN          SIG
  GROUP    IDENTIFICATION          MEAN          ORIGINAL UNITS    T STAT
0.05
  ----- --------------------    ----------    ------------------  ------  ---
    1            Neg Control     92.5000            92.5000
    2                    4.3     92.5000            92.5000        0.0000
    3                    9.1     96.2500            96.2500       -0.8367
    4                     16     86.2500            86.2500        1.3944
    5                     32     27.5000            27.5000       14.5021  *
    6                     59     12.5000            12.5000       17.8487  *
  ------------------------------------------------------------------------------
  Dunnett critical value = 2.3100   (1 Tailed, alpha = 0.05, df [used] = 5,40)
                                                              (Actual df = 5,42)


  Title:  Day 10 % Survival
  File:             6603s             Transform:             NO TRANSFORMATION
```

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	8			
2	4.3	8	10.3537	11.2	0.0000
3	9.1	8	10.3537	11.2	-3.7500
4	16	8	10.3537	11.2	6.2500
5	32	8	10.3537	11.2	65.0000
6	59	8	10.3537	11.2	80.0000

Title: Day 10 % Survival
File: 6603s Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg Control	8	92.5000	92.5000	93.7500
2	4.3	8	92.5000	92.5000	93.7500
3	9.1	8	96.2500	96.2500	93.7500
4	16	8	86.2500	86.2500	86.2500
5	32	8	27.5000	27.5000	27.5000
6	59	8	12.5000	12.5000	12.5000

Title: Day 10 % Survival
File: 6603s Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	92.5000				
4.3	93.7500	-0.2789		1.6800	k= 1, v=40
9.1	93.7500	-0.2789		1.7600	k= 2, v=40
16	86.2500	1.3944		1.7900	k= 3, v=40
32	27.5000	14.5021	*	1.8000	k= 4, v=40
59	12.5000	17.8487	*	1.8000	k= 5, v=40

s = 8.9642

WARNING: Procedure has used isotonized means which differ from original

(transformed) means.

Title: Day 10 Dry weight
File: 6603w Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Solvent cntl) Mean =	0.1012	Calculated t value =	1.0237
GRP2 (Blank cntl) Mean =	0.0950	Degrees of freedom =	14
Difference in means =	0.0062		

2-sided t value (0.05,14) = 2.1448 No significant difference at alpha=0.05
2-sided t value (0.01,14) = 2.9768 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Day 10 Dry weight
File: 6603w Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.0063
W = 0.9541

Critical W = 0.9040 (alpha = 0.01 , N = 32)
W = 0.9300 (alpha = 0.05 , N = 32)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Day 10 Dry weight
File: 6603w Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	3	0.0003	0.0001	1.0035
Within (Error)	28	0.0024	0.0001	
Total	31	0.0027		

(p-value = 0.4058)

Critical F = 4.5681 (alpha = 0.01, df = 3,28)
= 2.9467 (alpha = 0.05, df = 3,28)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Day 10 Dry weight
File: 6603w Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	3	0.0023	0.0008	3.3776
Within (Error)	28	0.0063	0.0002	
Total	31	0.0086		

(p-value = 0.0321)

Critical F = 4.5681 (alpha = 0.01, df = 3,28)
= 2.9467 (alpha = 0.05, df = 3,28)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Title: Day 10 Dry weight
File: 6603w Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Neg Control	0.1012	0.1012		
2	4.3	0.0938	0.0938	0.9990	
3	9.1	0.1025	0.1025	-0.1665	
4	16	0.0813	0.0813	2.6640	*

Dunnett critical value = 2.1700 (1 Tailed, alpha = 0.05, df [used] = 3,24)
(Actual df = 3,28)

Title: Day 10 Dry weight
File: 6603w Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Neg Control	8			
2	4.3	8	0.0163	16.1	0.0075
3	9.1	8	0.0163	16.1	-0.0013
4	16	8	0.0163	16.1	0.0200

Title: Day 10 Dry weight
File: 6603w Transform: NO TRANSFORMATION

William's Test - TABLE 1 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg Control	8	0.1012	0.1012	0.1012
2	4.3	8	0.0938	0.0938	0.0981
3	9.1	8	0.1025	0.1025	0.0981
4	16	8	0.0813	0.0813	0.0813

Title: Day 10 Dry weight
File: 6603w Transform: NO TRANSFORMATION

William's Test - TABLE 2 OF 2 Ho: Control<Treatment

IDENTIFICATION	COMPARED MEANS	CALC. WILLIAMS	SIG 0.05	TABLE WILLIAMS	DEGREES OF FREEDOM USED
Neg Control	0.1012				
4.3	0.0981	0.4163		1.7000	k= 1, v=28
9.1	0.0981	0.4163		1.7800	k= 2, v=28
16	0.0813	2.6640	*	1.8100	k= 3, v=28

s = 0.0150

WARNING: Procedure has used isotonized means which differ from original (transformed) means.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound
		Lower	Upper		/Estimate
EC5	4.3	1.1	16.	0.29	0.26
EC10	6.6	2.2	20.	0.24	0.33
EC25	14.	6.4	28.	0.16	0.48
EC50	30.	20.	46.	0.092	0.65

Slope = 1.94 Std.Err. = 0.534

!!!Poor fit: p < 0.001 based on DF= 3.00 39.0

6603WE : Day 10 Dry weight

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	8.00	0.101	0.105	-0.00354	100.	0.00
4.30	8.00	0.0937	0.0995	-0.00576	95.0	5.04
9.10	8.00	0.102	0.0884	0.0141	84.3	15.7
16.0	8.00	0.0812	0.0737	0.00759	70.3	29.7
32.0	8.00	0.0288	0.0502	-0.0215	47.9	52.1
59.0	5.00	0.0440	0.0299	0.0141	28.5	71.5

!!!Warning: EC5 not bracketed by doses evaluated.